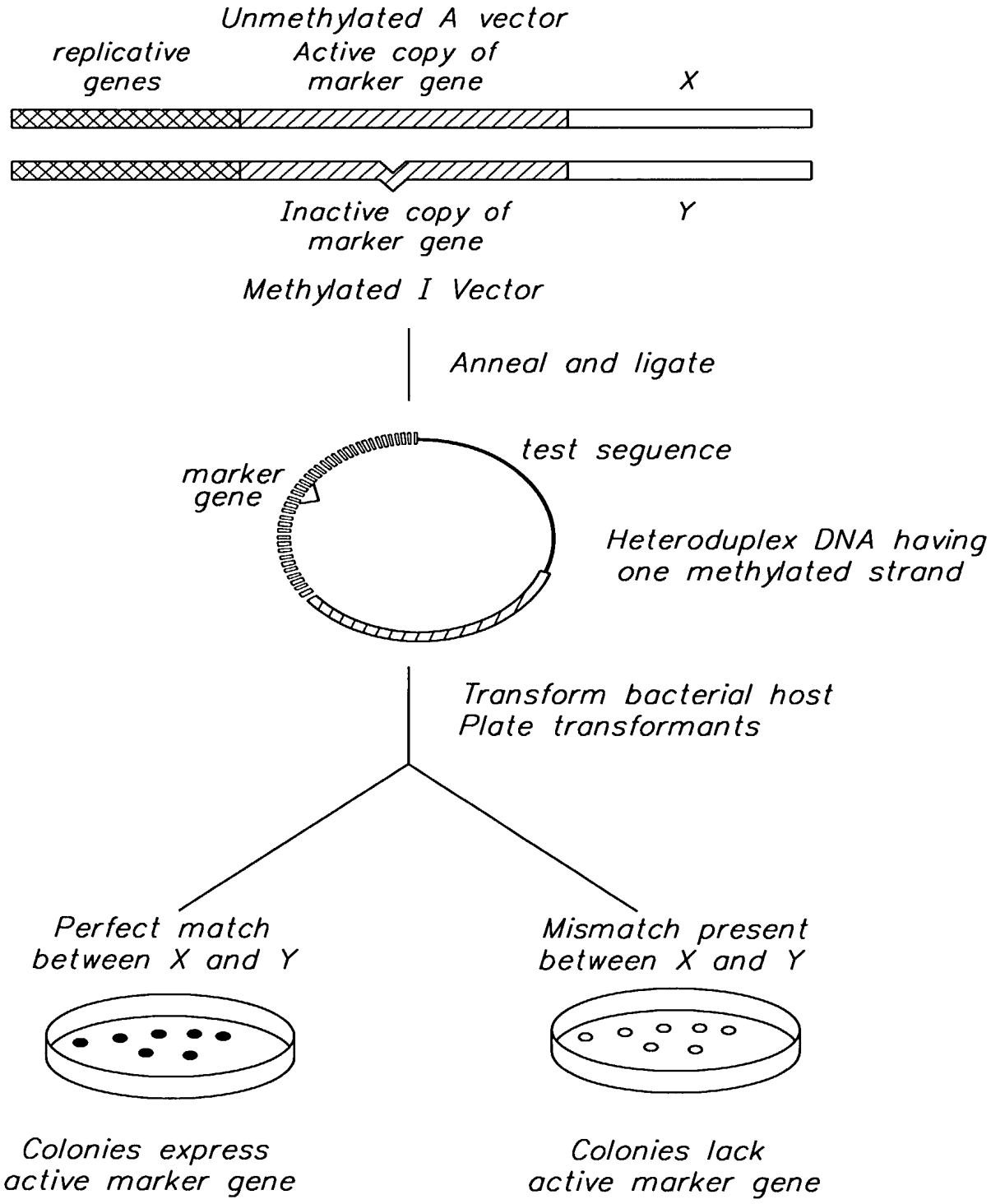

Unmethylated A vector
replicative genes
Active copy of marker gene
X
Inactive copy of marker gene
Y
Methylated I Vector
Anneal and ligate
marker gene
test seguence
Heteroduplex DNA having one methylated strand
Transform bacterial host
Plate transformants
Perfect match between X and Y
Mismatch present between X and Y
Colonies express active marker gene
Colonies lack active marker gene

FIG. 1

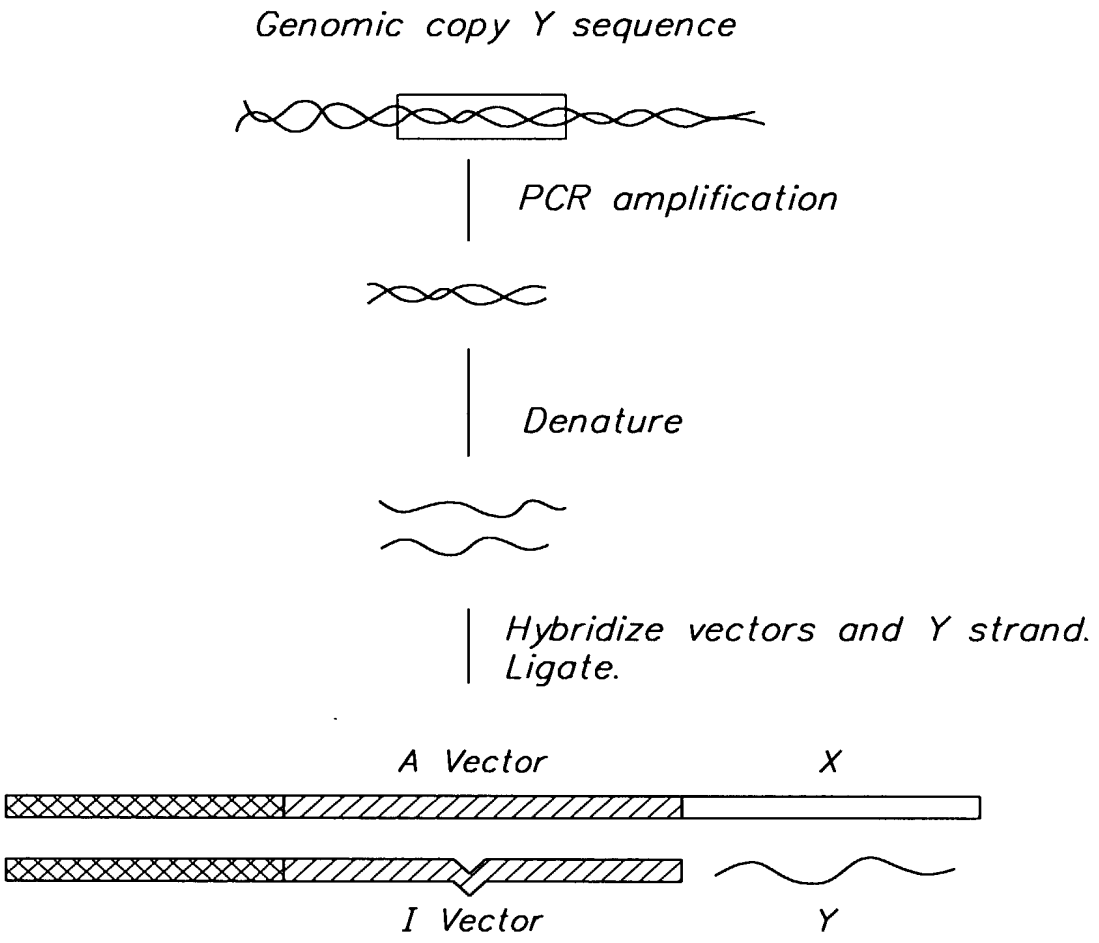

Genomic copy Y sequence
PCR amplification
Denature
Hybridize vectors and Y strand.
Ligate.
A Vector
X
I Vector
Y

FIG. 2

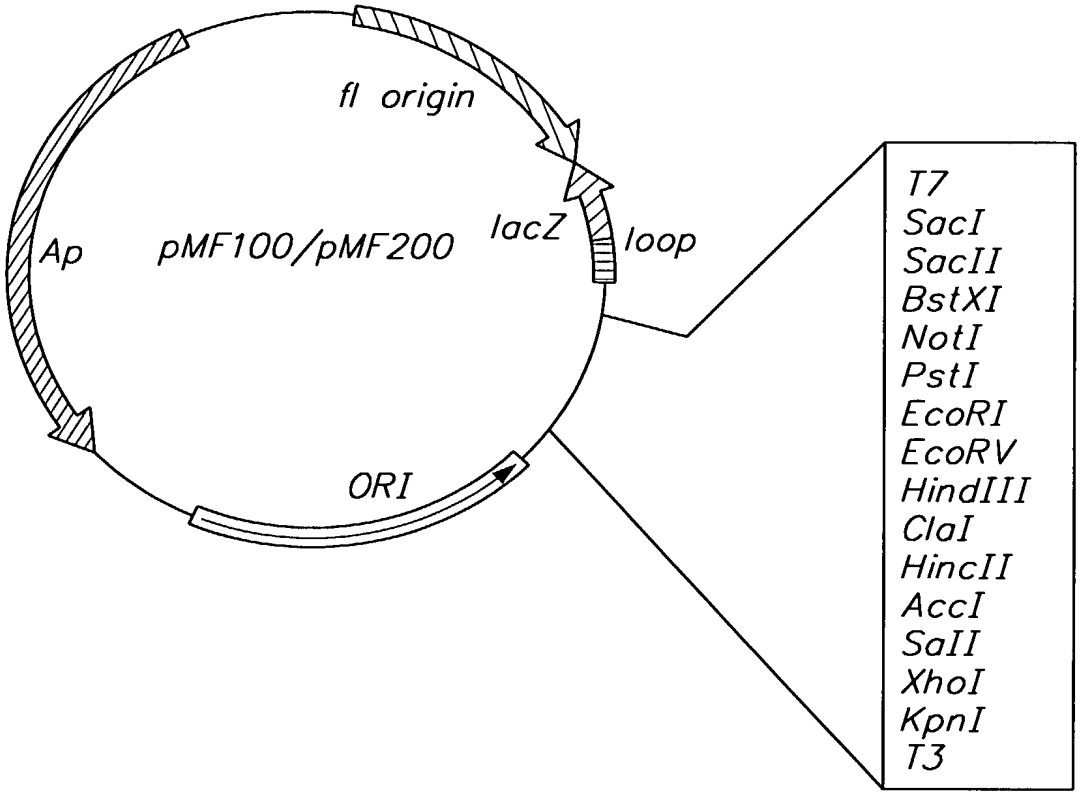
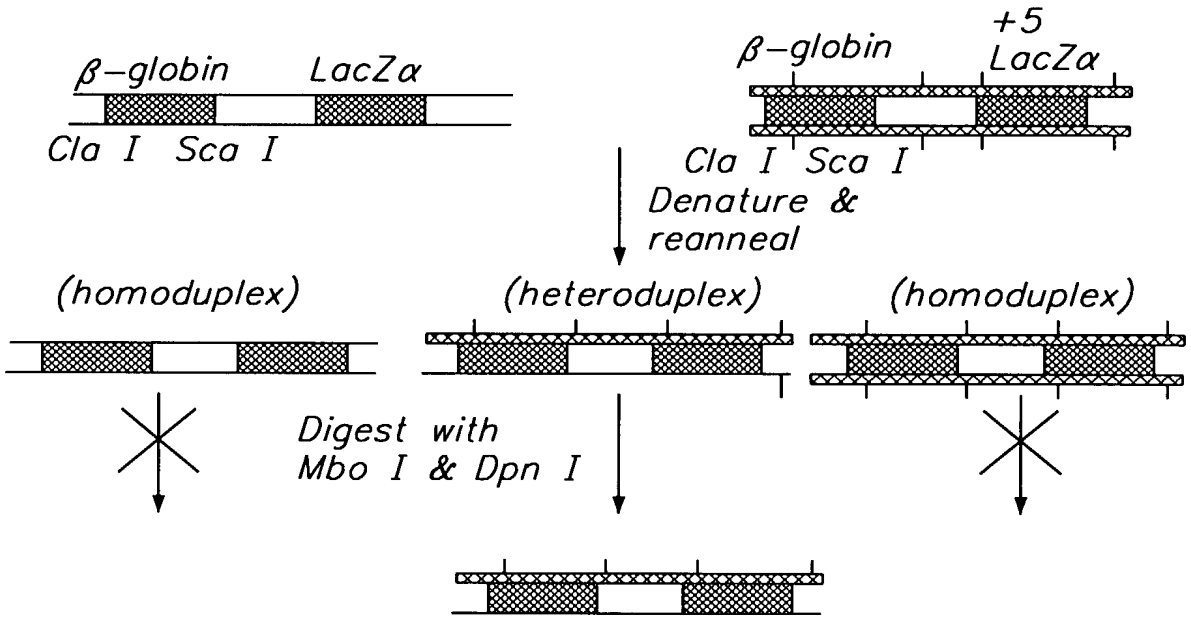

fl origin
Ap
pMF100/pMF200
lacZ
loop
ORI
T7
SacI
SacII
BstXI
NotI
PstI
EcoRI
EcoRV
HindIII
ClaI
HincII
AccI
SaII
XhoI
KpnI
T3

FIG. 3



| : methylated GATC
——— : plasmid with intact LacZα
▭ : plasmid with a 5 bp insertion in LacZα

FIG. 4

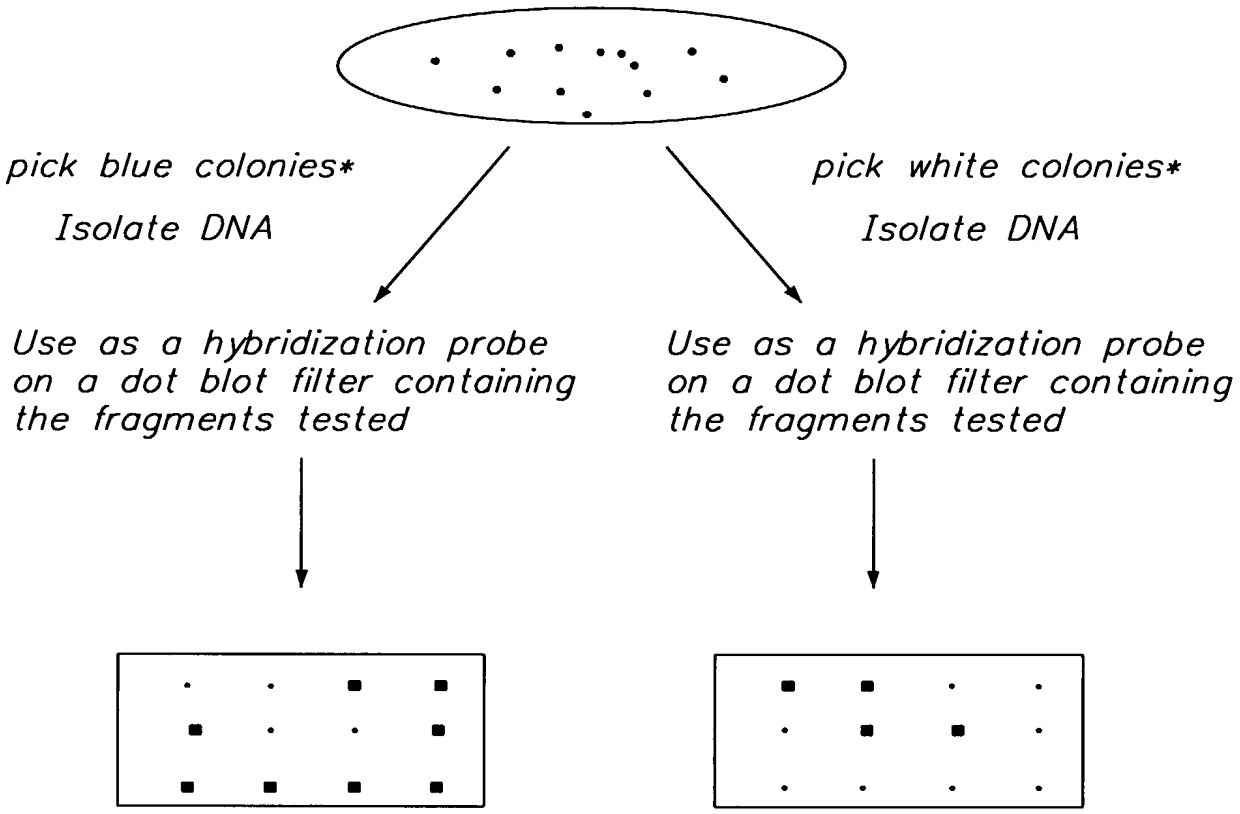

pick blue colonies*
Isolate DNA
pick white colonies*
Isolate DNA
Use as a hybridization probe on a dot blot filter containing the fragments tested
Use as a hybridization probe on a dot blot filter containing the fragments tested
comparing the hybridization signal on both filters, one can determine which fragments are variant

FIG. 5

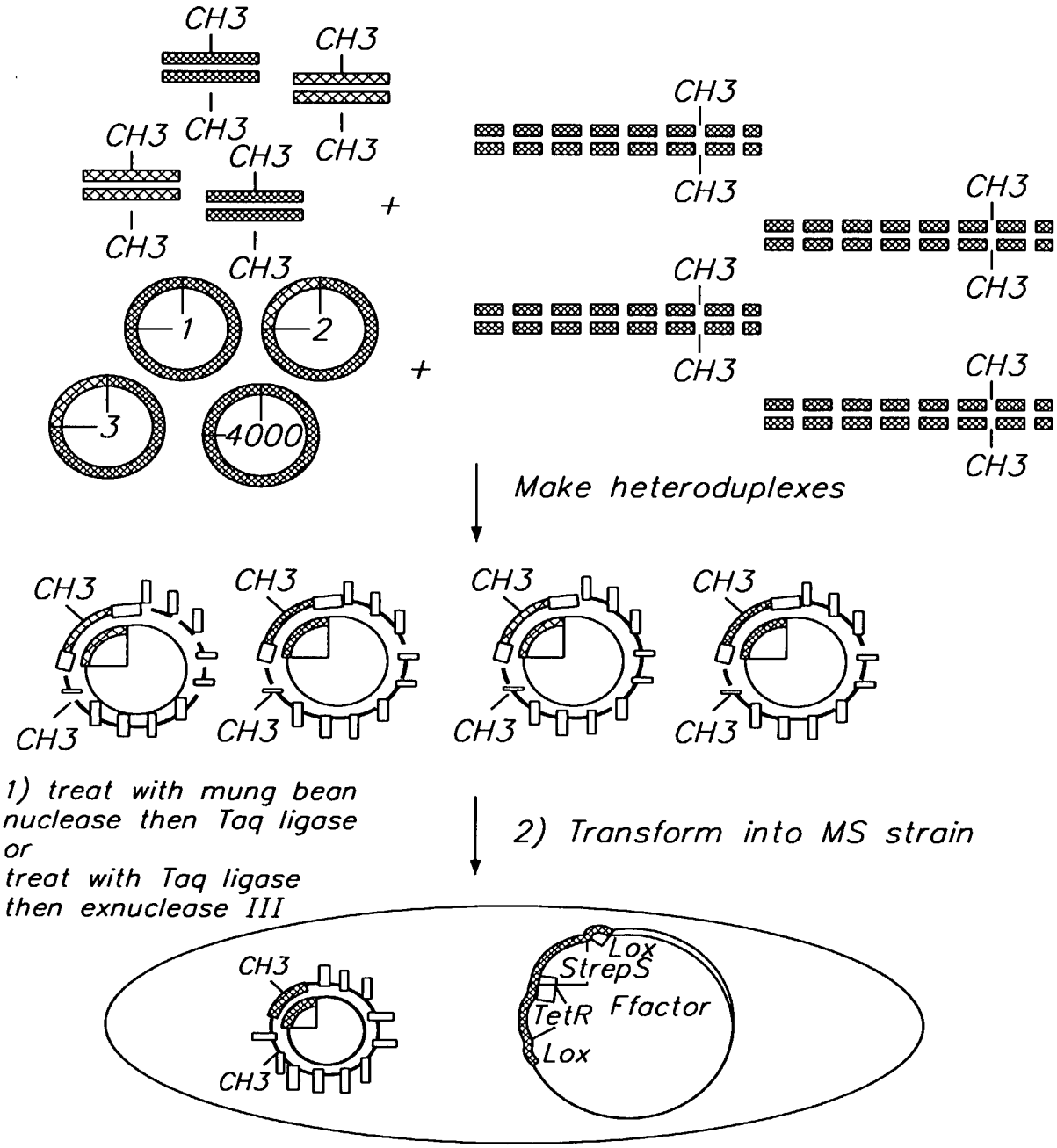
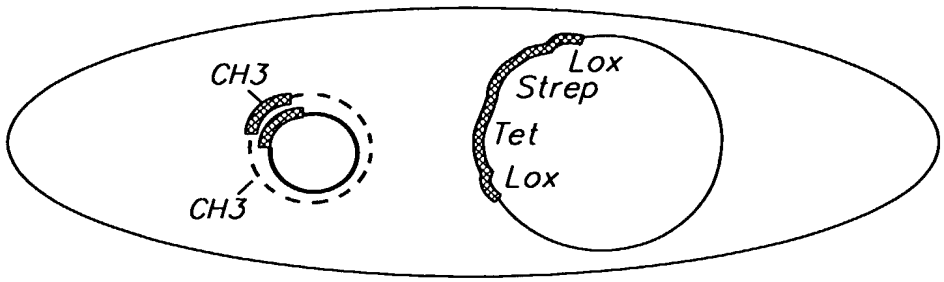
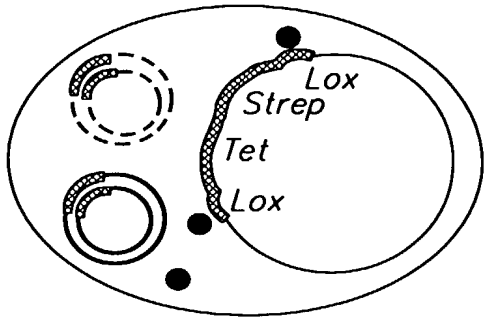

CH3
1
2
3
4000
Make heteroduplexes
1) treat with mung bean nuclease then Taq ligase or treat with Taq ligase then exnuclease III
2) Transform into MS strain
Lox
StrepS
TetR
Ffactor
Lox

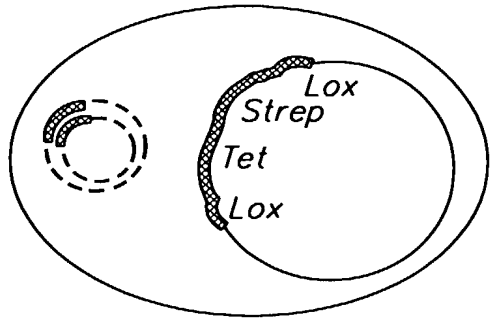
FIG. 6A



In absence of a variation, no repair occurs. Both strands are replicated

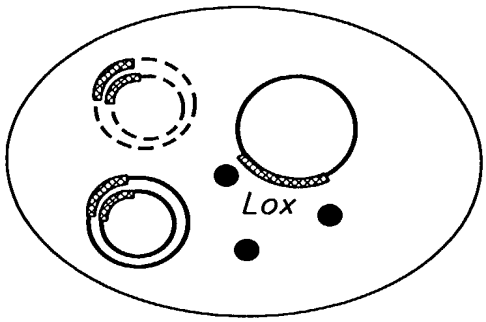
In presence of a variation, repair occurs. Only the strand w/inactive Cre is replicated

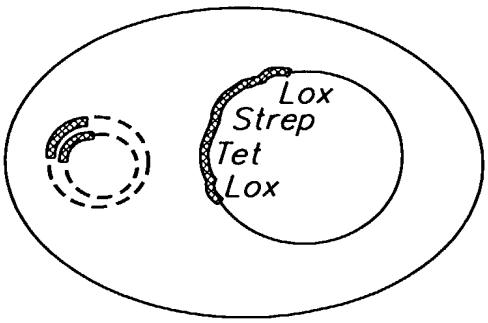




Active Cre is present in the cell

Active Cre is absent in the cell





Cell is Tet sensitive & Strep resistant

Cell is Tet resistant & Strep sensitive

FIG. 6B

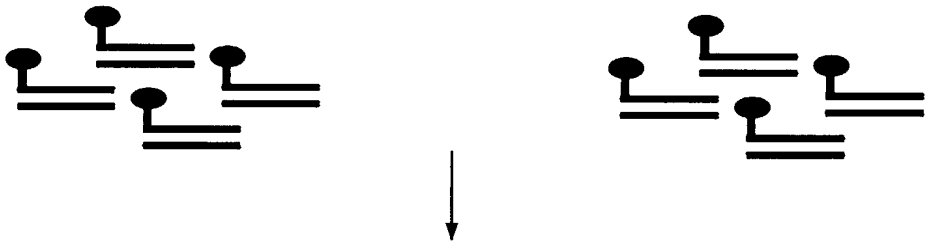
Cells are grown in two tubes supplemented either with

Tetracycline or Streptomycin

Next day DNA is preped from the pool of the cells grown in each tube

DNA from the Tet pool is labeled with green fluorescence

DNA from the Strep pool is labeled with red fluorescence



DNA from both pools are mixed and hybridized to a DNA microarray.
Each spot corresponds to a different gene fragment that is being tested

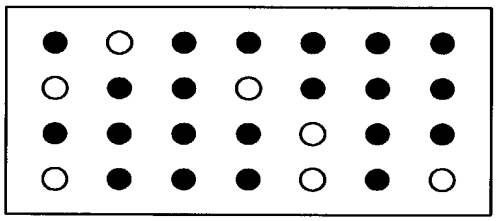


FIG. 6C

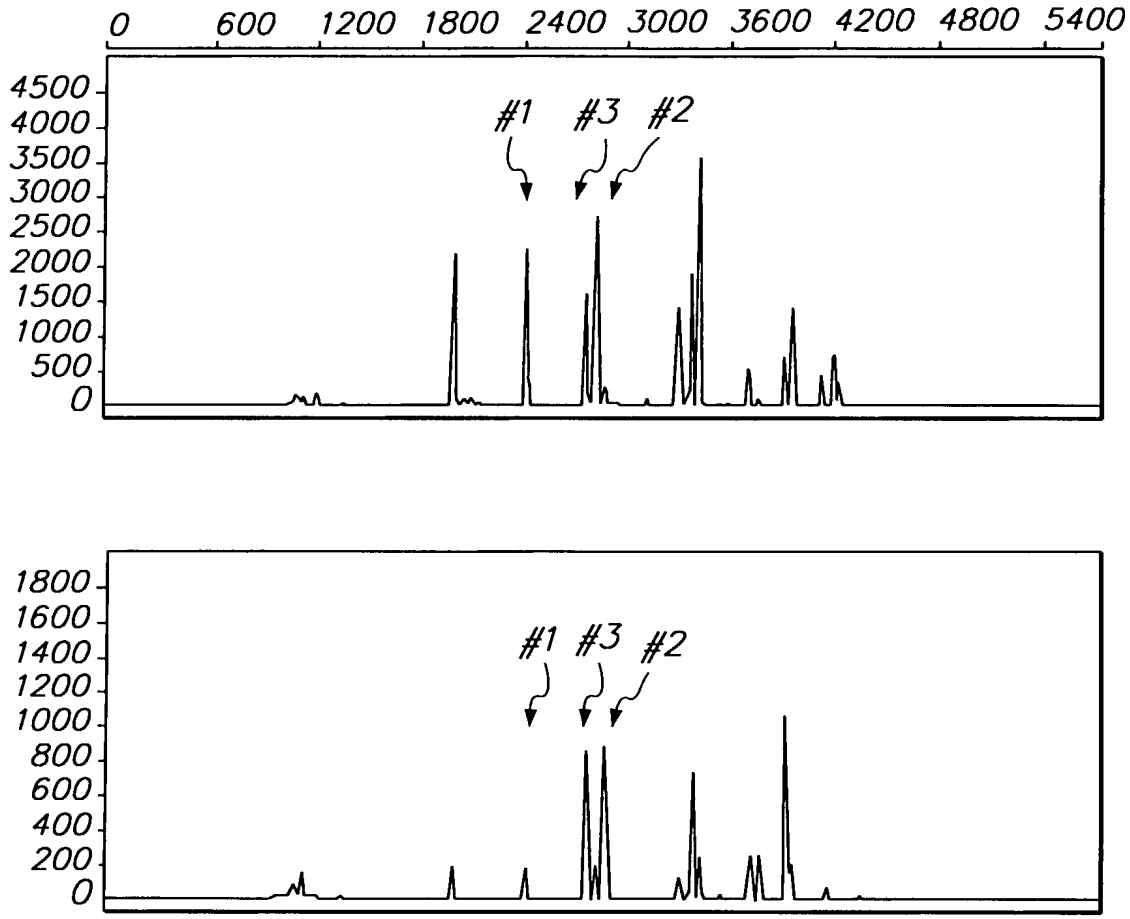

0
600
1200
1800
2400
3000
3600
4200
4800
5400
4500
4000
3500
3000
2500
2000
1500
1000
500
0
#1
#3
#2
1800
1600
1400
1200
1000
800
600
400
200
0
#1
#3
#2

FIG. 7